# Low Doses of UVB or UVA Induce Chromosomal Aberrations in Cultured Human Skin Cells

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**Chromosomal defects are frequently present in malignant and premalignant skin disorders; however, it is not known whether ultraviolet radiation from sunlight plays a role in their induction. To obtain information on the ability of ultraviolet A and ultraviolet B to induce chromosomal aberrations, cultured melanocytes and fibroblasts were exposed to physiologic doses of ultraviolet A or ultraviolet B and, for comparison, to γ rays. As a measure of chromosomal aberrations, the formation of micronuclei was determined. To obtain sufficient statistical data on induced micronuclei and cell kinetics, a flow cytometry method has been modified and applied. The flow cytometry method analysis is based on staining the DNA with ethidium bromide and the cell membranes with 1,6-diphenyl-1,3,5,-hexatriene. We observed dose-dependent micronuclei formation after γ or ultraviolet B irradiation in both cell types and also for ultraviolet A in fibroblasts. The yield of micronuclei induced in fibroblasts by ultraviolet A was only a factor 15 smaller than that induced by ultraviolet B (313 nm). The results indicate that 10 kJ per $m^2$ (equivalent to 1 minimal erythema dose) of ultraviolet B and 150 kJ per $m^2$ of ultraviolet A (0.2 minimal erythema dose) can induce 1% of micronuclei in fibroblasts, equivalent to the induction due to 0.6 Gy of γ radiation. In conclusion, physiologic doses of sunlight can induce chromosomal aberrations at a level comparable with that observed after exposure to approximately 1 Gy of ionizing radiation. Therefore, sunlight can be considered a potential inducer of chromosomal aberrations in skin cells, which may contribute to skin carcinogenesis.** ***Key words: human fibroblasts/human melanocytes/micronuclei/skin cancer. J Invest Dermatol 115:435–440, 2000***

The incidence of human cutaneous malignancies is related to the sun exposure of human skin (Mukhtar and Elmets, 1996). This relationship is rather convincing for nonmelanoma skin cancers, whereas melanoma development appears to be more complicated (Weinstock, 1996; Gilchrest *et al*, 1999). The etiology of skin cancer has been described as a multistep process (Yuspa and Dlugosz, 1991) in which ultraviolet A (UVA) as well UVB play a role (Matsui and DeLeo, 1991). The role of UVA is supported by data concerning tumor induction in hairless albino mice (De Gruijl *et al*, 1993) and even by melanoma in fish (Setlow *et al*, 1993).

Molecular analyses of tumors have demonstrated that a whole spectrum of genetic modifications can occur in skin cancer cells (Heim and Mitelman, 1995; Leis and Livingstone, 1996). In addition to point mutations and small deletions, the loss of an entire gene, a chromosome fragment, or a whole chromosome may also result and can be detected cytogenetically (Solomon *et al*, 1991). Distinct patterns of chromosome loss were found in basal cell carcinoma and squamous cell carcinoma of human skin (Quinn *et al*, 1994); moreover, a high frequency of loss of heterozygosity in actinic keratoses has been reported (Rehman *et al*, 1994). In addition, serious chromosomal aberrations have been observed in the melanoma cells of human skin (Dracopoli *et al*, 1989; Trent *et al*, 1989). Several genetically toxic alterations that may lead to skin carcinogenesis have been described (Yunis, 1983; Rees, 1994), some of which are known to be induced by UV light (IARC, 1992). It is still not known, however, which part of the UV spectrum causes these defects and to what extent chromosomal aberrations are induced by UV irradiation.

A very sensitive parameter for induced cytogenetic modifications that is indicative of chromosomal damage is the induction of micronuclei (MN; Heddle *et al*, 1983). MN represent genetic material that cannot be incorporated into daughter nuclei at the time of cell division (Cornforth and Goodwin, 1991). These round particles are surrounded by an envelope composed of inner and outer membranes (Paglin *et al*, 1997). It is thought that MN result from DNA double-strand breaks (dsb) possibly caused by repair of UV-induced pyrimidine dimers (Bradley, 1981) or induced by radicals formed after ionizing radiation. In previous studies, MN induction caused by solar UV was found to be higher in cultured human fibroblasts from xeroderma pigmentosum (Bielfeld *et al*, 1989) or familial melanoma patients (Roser *et al*, 1989) than in cells derived from healthy

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Abbreviations: dsb, double-strand breaks; DPH, 1,6-diphenyl-1,3,5-hexatriene; FCM, flow cytometry; FM, fluorescence microscopy; MN, micronuclei.

individuals, in accordance with the enhanced cancer risk of these patients from sun exposure.

The goals of this study were (a) to investigate the MN-inducing capacity of experimental and clinically relevant doses of UVB and UVA *versus* γ rays in human fibroblasts as well as in melanocytes; and (b) to adapt a multiparameter flow cytometry method (FCM) (Wessels and Nüsse, 1995) to measure MN formation and simultaneously obtain cell cycle data in our cell types.[1] Our results indicate that low doses of UVB and UVA induced approximately one MN per 100 nuclei, a level equal to the MN induction potential of 0.6 Gy of ionizing radiation.

## MATERIALS AND METHODS

**Isolation and culture of human melanocytes and fibroblasts** Cells were isolated from neonatal foreskin (skin type II) by a modification of the method described by Liu and Karasek (1987). Briefly, foreskins were freed from connective tissue, cut into small pieces, and incubated in a trypsin solution (0.25% trypsin, 0.15 M NaCl, 0.04 M KCl, 0.1% glucose, pH 7.5) overnight at 4°C. The skin fragments were transferred into growth medium and the epidermis and dermis were separated.

For melanocyte cultures, the epidermal cells obtained were grown according to the method of Eisinger and Marko (1982), as modified by Halaban *et al* (1986). The basis of the culture medium was Ham's F-10 supplemented with 10 nM 12-O-tetra-decanoyl-phorbol-13-acetate, 2.5 nM choleratoxin, 0.1 mM isobutylmethylxanthine, 5% fetal bovine serum (Hyclone, Logan, UT), 100 IU penicillin per ml, and 100 μg streptomycin per ml. If cultures were contaminated with fibroblasts, geneticin (100 μg per ml) was added to the growth medium for 48–72 h. Fibroblasts obtained from the dermis were cultured in Ham's F-10 supplemented with 10% newborn calf serum, 100 IU penicillin per ml and 100 μg streptomycin per ml. Cells were cultured at 37°C in a 5% $CO_2$ atmosphere. The growth medium was refreshed twice a week. Cell growth was maintained in log-phase as evaluated by phase-contrast microscopy. Cultured cells between passages 2 and 9 were used for experiments.

**Irradiation and dosimetry** UVB radiation was provided by three custom-made 40 W Philips TL-01 fluorescent tubes (Philips Nederland, Eindhoven, The Netherlands), emitting mainly UVB of 313 nm (Sterenborg *et al*, 1988). The incident dose rate was 3 W per $m^2$. During UVB exposure, the covers of the Petri dishes were removed. For UVA irradiation, a Sellas Sunlight lamp (type 2001, Sellas, Gevelsberg, Germany) was used, which was equipped with UVB filters (UVASUN blue sheet and UVASUN blue film) to exclude transmission below 340 nm. The emission spectrum of the light after it passed through the filters was determined by means of an optronic spectroradiometer (Philips Drachten, The Netherlands), shown in **Fig 1.** The dose rate under the cover of the dish was 70 W per $m^2$. The fluence was monitored with an IL-700 research radiometer (International Light, Newburyport, MA) equipped with a SEE 400 detector in combination with a WBS 320 filter. The $^{60}Co$ γ irradiation was performed at a dose rate of 3.3 Gy per min. The control cultures were kept under identical conditions and protected from radiation.

**MN induction** Exponentially growing cells were seeded on glass slides, for fluorescence microscopy (FM) scoring, and in culture dishes, for FCM analysis, at a density of $4 \times 10^3$ cells per $cm^2$. Fibroblasts and melanocytes were irradiated 24 h and 72 h later, respectively, in monolayers covered with phosphate-buffered saline. After irradiation, the phosphate-buffered saline was replaced by the appropriate growth medium and the cells were further incubated at 37°C in a 5% $CO_2$ atmosphere. FM and/or FCM analyses were performed 4 d or 8 d after irradiation for fibroblasts or melanocytes, respectively.

**Detection of MN by FM** Glass slides with cultured cells were washed in phosphate-buffered saline, treated with a hypotonic solution (0.1 M NaCl, 1.7 mM KCl), and fixed with methanol:acetic acid:phosphate-buffered saline (1:3:4). The DNA of the nuclei and MN was stained with propidium iodide at a concentration of 5 μg per ml in Vectashield (Vector Laboratories, U.S.A.). Within intact interphase cells, the particles that were observed to be completely separated from the main nucleus were scored as MN. Their diameters ranged between an eighth and a half of the diameter of the nucleus (Cornforth and Goodwin, 1991). At least 1000 cells were scored on coded slides.

[1]Emri G, *et al*: *Cytometry* S9:87, 1998 (abstr.)

**Preparation and staining of cells for FCM analysis** A suspension of nuclei and MN was prepared using a modified method described by Wessels and Nüsse (1995). Briefly, 0.5 ml Solution I containing 10 mM NaCl, 3.4 mM Na citrate, 10 mg per l RNase A (from bovine pancreas), and 0.03% (vol/vol) Nonidet p-40 was added to the pellets of $(3–6) \times 10^5$ cells, followed by the addition of ethidium bromide (EB) (Sigma) at a final concentration of $1.3 \times 10^{-5}$ M. After 1.5 h incubation at room temperature in the dark, 0.5 ml Solution II containing 71.4 mM citric acid, 0.25 M sucrose, and 2 mM ethylenediamine tetraacetic acid was added. The samples were stored at 4°C until the analysis was performed the next day. Cellular membranes were stained with the fluorescent dye 1,6-diphenyl-1,3,5,-hexatriene (DPH) (Sigma), which was added 3–4 h before measurements to a final concentration of $1.1 \times 10^{-4}$ M.

**FCM analysis and sorting** The measurements were carried out with an Epics Elite (Coulter, Luton, U.K.) flow cytometer equipped with two $Ar^+$ ion lasers tuned at 488 nm and 351-363 nm for the excitation of the fluorochromes EB and DPH, respectively. For excitation of the two fluorochromes by the two lasers, a time delay (≈40 μs) was applied, based on the temporal separation of the two laser beams. The arrival of signals from the first laser at the computer was delayed in order to coincide with the arrival of signals from the second laser. The optical filters used for EB and DPH fluorescence were, respectively, a long wavelength pass filter at 630 nm and a 450 nm bandpass filter in combination with a long pass dichroic mirror at 550 nm. The mean EB fluorescence intensity of $G_1$-phase nuclei was set at 2000 fluorescence units. A trigger was set at 0.05% of the EB fluorescence of $G_1$ nuclei. The data were recorded in listmode by the Elite software. A total of 1500 particles were evaluated from bivariate log DPH *versus* log EB plots using a gate including MN and nuclei for each sample (6000–12,000 total events). Additionally, the gated events were further analyzed using the pulse area and peak signals of the EB fluorescence correcting for doublets (Bauer and Boezeman, 1983) to detect the cell cycle progression of the main nuclei. Particles with certain EB and DPH fluorescence intensity were sorted, collected on glass slides (500–1000 events), and identified as nuclei, MN, or debris by FM.

**Dose–response analysis** Percentages of MN and $G_2$/M-phase nuclei were determined in the cell cultures. Percentage of $G_2$/M-phase nuclei was calculated as the proportion of particles with doubled DNA content of $G_1$-phase nuclei. Subtracting the background values of MN induction (sham irradiation), the mean values of three to five independent experiments and the standard error of the mean were plotted as a function of dose. Regression analyses of data were carried out by Microsoft Excel 5.0a software for Windows 95. Statistical significance was calculated using the Student's *t* test ($p \leq 0.05$).

## RESULTS

**Modification of the dual laser FCM method made it possible to measure MN in cultured human skin cells** To distinguish between the particles of interest (nuclei, MN) and nonspecific cellular debris, a gate was established in the dot plot of DPH *versus* EB fluorescence intensity (**Fig 2*a*, *b***). Organelles with lower DPH fluorescence and with a relative DNA content between 1% and 20% of $G_1$-phase nuclei (**Fig 2*c*, *d***) were identified as MN by sorting. The following modifications were introduced to the method described by Wessels and Nüsse (1995): (i) a longer incubation time (1.5 h) to establish improved separation of the MN and nuclei from the cytoplasm after cell lysis; (ii) addition of ethylenediamine tetraacetic acid (1 mM) to the free MN after cell lysis to protect their DNA from endonucleases; and (iii) applying a time delay to the dual laser FCM measurement (see also *Materials and Methods*).

**Cultured fibroblasts and melanocytes need a post-irradiation incubation time of 4 and 8 d, respectively, to develop MN** First, we determined the post-irradiation period required for the development of MN. For dose dependence studies, we measured the formation of MN at the point when the MN frequency reached its plateau for the highest dose applied. In all of our experiments, UVB, UVA, and γ radiation doses did not cause more than 50% cell killing (see *Discussion*). In melanocytes, however, MN were detectable up to a dose of 9 Gy. In our experiments using microscopic

determination of MN, we found that 4 d incubation time was required after irradiation for fibroblasts and 8 d for melanocytes to obtain an optimal MN frequency.

**Gamma and UVB radiation induce MN and cell cycle arrest in cultured fibroblasts and melanocytes in a dose-dependent way** We found a sharp increase in the induction of MN after γ irradiation with doses up to approximately 9 Gy in human melanocytes and up to 4 Gy in fibroblasts (**Fig 3*a***). The increment of the proportion of $G_2$/M-phase nuclei accompanying the same dose was also higher in fibroblasts than in melanocytes (**Fig 3*a***). We detected linear dose responses for MN induction in fibroblasts for UVB doses up to 11 kJ per $m^2$ (**Fig 3*b***). Higher doses of UVB (up to 20 kJ per $m^2$) induced approximately the same percentage of MN in human melanocytes (**Fig 3*b***). Maximum MN frequencies were $1.27 \pm 0.24\%$ and $1.1 \pm 0.24\%$ in fibroblasts and in melanocytes, respectively. The percentage of cells in $G_2$/M-phase also showed a dose-dependent increase in both cell types, but the increment accompanying the same dose was higher in fibroblasts than in melanocytes (**Fig 3*b***).

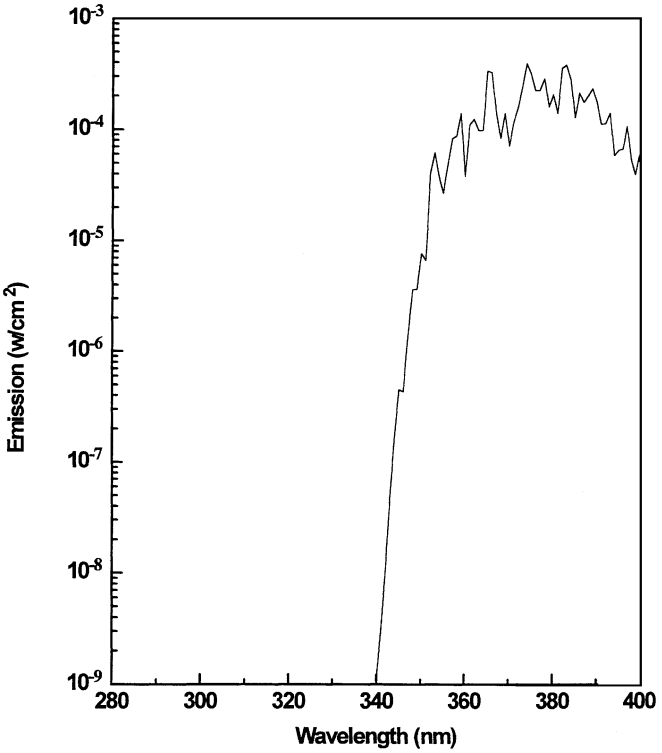


**Figure 1. Spectral composition of the UVA after passing the applied filters.**

**MN and cell cycle arrest are induced in cultured fibroblasts after irradiation with UVA** For comparison and validation of the FCM method, we determined the MN induction by means of FM. A linear dose–response relationship was obtained for the formation of MN in fibroblasts after irradiation with doses of UVA up to 300 kJ per $m^2$ (**Fig 4**). The maximum MN frequency amounted to approximately twice the control value ($p \leq 0.05$). The induction level observed for MN was higher when scored by FM than by FCM. The proportion of the cells in $G_2$/M-phase also increased in a dose-dependent manner up to approximately 20%, as detected by FCM (**Fig 4**). We did not observe any induction of MN nor delay of cell cycle in melanocytes irradiated with UVA up to doses of 300 kJ per $m^2$.

## DISCUSSION

To determine MN induction with FCM, we first modified a previously employed multiparameter FCM method (Wessels and Nüsse, 1995) for the cell types under study. We obtained a dose response of MN and cell cycle delay induction in melanocytes and fibroblasts after irradiation with low doses of UV or with γ rays. The induced MN represent an endpoint after the first cell division following irradiation, when various repair processes have already taken place. Therefore, a certain time period after irradiation is required to allow mitotic processes. This time period is dependent on the cell cycle time as well as on the MN inducing agent (Geard and Chen, 1990). The MN detection method had to be adapted for melanocytes and fibroblasts, which have cell cycle time durations of 31 h (De Leeuw *et al*, 1994) and 12 h (Kaufmann and Wilson, 1994), respectively. An 8 d post-irradiation time period was required for melanocytes, whereas 4 d were needed for fibroblasts to obtain optimal MN frequencies (data not shown). In addition, we adapted the method with regard to the staining of the cells and we modified the FCM measurements, including the gate settings appropriate for MN (**Fig 2**). An overlapping area remained between MN and debris as determined by sorting followed by inspection with FM. Therefore, setting a gate to exclude the debris resulted in exclusion of some MN from further calculations. This is a likely explanation for why the frequency determined for MN was lower by FCM than by FM (**Fig 4**). This phenomenon has also

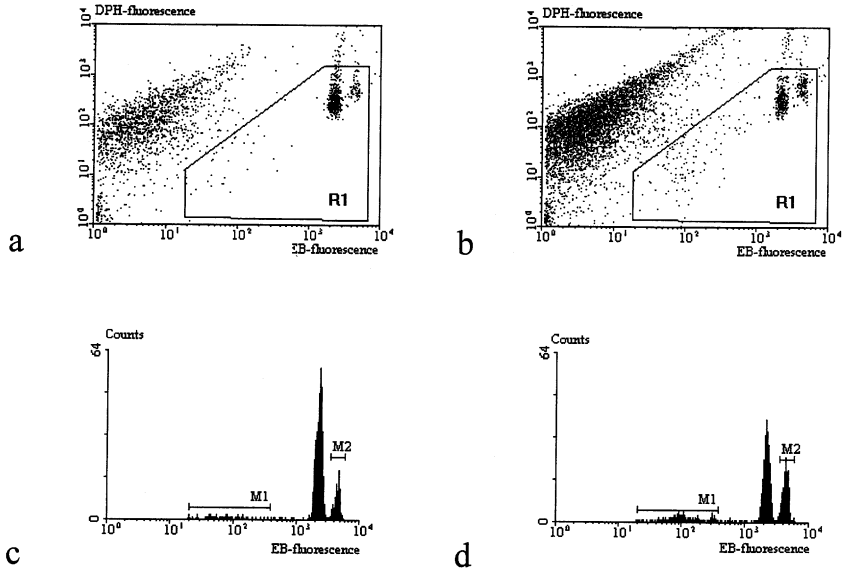


**Figure 2. FCM measurements showing increased numbers of MN and G2/M arrest in human fibroblasts after exposure to 4 Gy of γ rays or sham irradiation.** The dot plots (*a*) (sham irradiation) and (*b*) (4 Gy) show DPH fluorescence intensity (relative content of membranes) as a function of EB fluorescence intensity (relative DNA content) using logarithmic scales. The window (R1) shows the discrimination of nuclei and MN from debris as determined by sorting. A total of 1500 particles were evaluated within this window for each sample (6000–12,000 total events). The histograms (*c*, *d*) show the DNA distribution of particles within the window (R1). The number of particles is plotted as a function of EB fluorescence intensity (logarithmic scale). Particles (M1) with a relative DNA content between 1% and 20% of $G_1$-phase nuclei were identified as MN by sorting. The second nucleus peak (M2) represents the $G_2$/M-phase nuclei.

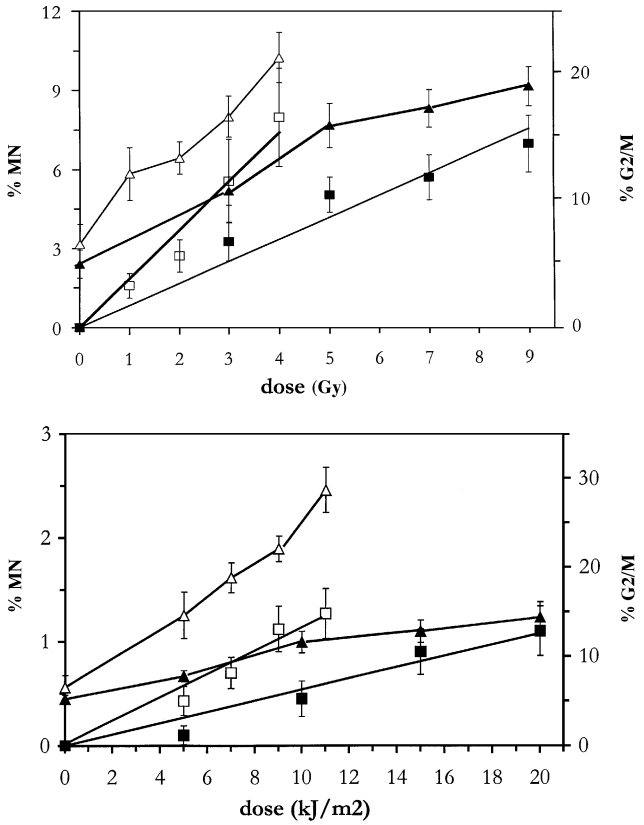


**Figure 3. Gamma irradiation as well as UVB irradiation induces MN and G2/M delay in a dose-dependent fashion in human fibroblasts and melanocytes.** Induction of MN in fibroblasts (□) and melanocytes (■) by γ radiation (*a*) and UVB radiation (*b*). In (*a*) the slopes for fibroblasts and melanocytes are 1.99 ± 0.19 and 0.75 ± 0.06, respectively; in (*b*) these values are 0.12 ± 0.01 and 0.06 ± 0.01. Percentage of $G_2$/M-phase nuclei was established from the same cell cultures of fibroblasts (△) or melanocytes (▲) by FCM as described in *Materials and Methods* and shown in **Fig 2**. Each symbol represents the mean frequency ± SEM of four to five independent experiments. The slopes were obtained by linear regression analysis of all data points. Sham values were subtracted in each experiment. These values were 1.5 ± 0.3 for fibroblasts and 1.0 ± 0.3 for melanocytes.

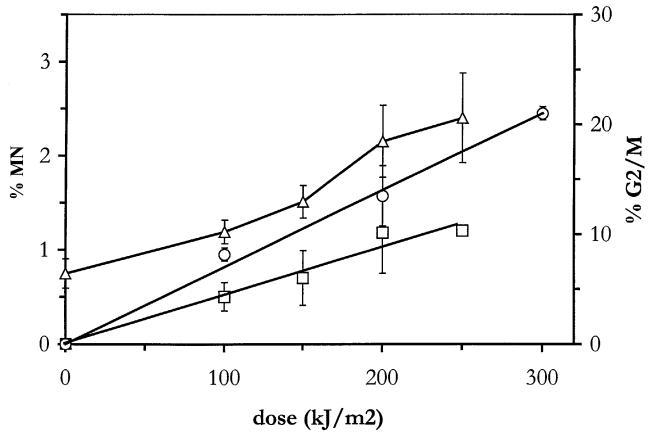


**Figure 4. Broadband UVA irradiation induces MN in human fibroblasts in a dose-dependent fashion as determined by FM and FCM, and a cell cycle delay was also determined.** Comparison of the sensitivity of FCM (□) and FM (○) MN assay. The percentage of MN measured in sham-irradiated cells (1.5 ± 0.3 in fibroblasts) has been subtracted. The slopes of the FCM and FM obtained data amount to 0.05 ± 0.001 and 0.007 ± 0.001, respectively, and were obtained by linear regression analysis of all data points. Percentages of $G_2$/M–phase nuclei (△) were established by FCM at the time of measurement of MN in the same samples. Each point represents the mean ± SEM of three to five separately performed experiments.

been found in other studies (Weller *et al*, 1996). Although Nüsse and Marx (1997) found that apoptotic bodies, eventually present in cell debris of some investigated cell types, cannot be gated out, in our samples, as indicated by the absence of a sub-$G_1$-phase (**Fig 2**), which is a clear indicator of apoptosis (Nüsse and Marx, 1997), no apoptotic cells were present in the sham and irradiated cells at the time of harvesting. Therefore, an overlap of the MN measurements by apoptotic bodies can be excluded. By applying the multi-parameter FCM, we were able to determine cell cycle arrest simultaneously with MN induction, in this case the $G_2$/M delay (**Figs 2–4**). Cell cycle arrest is described by several authors as an active physiologic response to DNA damage caused by, for example, γ rays (Kastan *et al*, 1991) or UV irradiation (Kusewitt *et al*, 1992). It is thought that this growth arrest enables the repair of induced DNA lesions more extensively prior to the next mitosis (Kaufmann and Kaufman, 1993; Bologna *et al*, 1994). Thus, cell cycle delay functions as a checkpoint to preserve a cell's genetic integrity. The effect of cell cycle arrest in relation to the induction of MN was demonstrated by Kaufmann and Wilson (1994). In fibroblasts derived from patients with ataxia teleangiectasia, which show multiple defects in activating the cell cycle checkpoints in response to ionizing-radiation-induced DNA damage (Shackelford *et al*, 1999), more MN were found than in cells derived from healthy people (Kaufmann and Wilson, 1994). Moreover, Weller *et al* (1996) found more MN in UV-irradiated cells if the cell cycle delay mechanism was overruled by the addition of caffeine. Our data on $G_2$/M delay induced by γ rays (**Fig 3*a***) are consistent with the underlying mechanism of MN induction, in this case the induction of DNA dsb (Burns and Sargent, 1981; Van der Schans *et al*, 1983). MN induced by UVB are thought to arise from DNA dsb as well. It is likely that these dsb are generated during excision repair processes (Bradley, 1981), however, or from dsb directly induced by UV as described by Peak and Peak (1990). In this study, we also presented MN induction by γ rays in human skin cells (**Fig 3*a***) because the induction of MN by γ rays in relation to cell killing has been studied in human fibroblasts (Geard and Chen, 1990) and in other cell types (Schreiber *et al*, 1992). This made it possible for us to compare the MN-inducing effects of *in vitro* and *in vivo* applied UV doses with experimental (Yuspa and Dlugosz, 1991) as well as radiotherapeutically applied doses of ionizing radiation (Traenkle, 1963; Van Vloten *et al*, 1987). In our experiments, we employed the same cell type (fibroblasts) and γ dose; moreover, the dose rate was also in the same range (**Fig 3*a***) as that described by Geard and Chen (1990). Our cell cultures were not trypsinized after irradiation, however, to ensure that the physiology of the cells was not disturbed, and that the conditions were optimal for DNA repair processes. This difference in protocol may explain the lower frequency for induced MN detected in our study. The values presented in **Fig 3(*a*)** and **3(*b*)** demonstrate a much lower induction of MN in melanocytes by γ and UVB radiation than in fibroblasts. This result may be due to the longer cell cycle of the melanocytes. A low number of induced MN was also found by Schreiber *et al* (1992) after γ irradiation in other cells with a long cell cycle. One explanation for the underlying mechanism is that the sensitivity of the cells for MN induction by γ rays is cell cycle dependent (Wolff, 1968); also the clastogenic effect of UV radiation seems to be S-phase dependent (Kaufmann and Wilson, 1994). The duration of S phase is the same in all cell types and is independent of cell cycle time. Thus, cell cultures with a long cell cycle have a lower percentage of cells in S phase than those with a short cell cycle (De Robertis and De Robertis, 1987).

Until recently there have been very few data available about UVB-induced MN in normal human fibroblasts (Krepinsky *et al*, 1980; Roser *et al*, 1989) and in modified human keratinocytes (Weller *et al*, 1996). Binucleated cells and MN were detected after UVA irradiation of hamster and mouse 3T3 cells (Bånrud *et al*, 1999), whereas in photosensitized human keratinocytes, MN were

detected after irradiation with visible light (Pflaum *et al*, 1998). In the experiments presented here, fibroblasts and melanocytes were irradiated with the same UVB source (313 nm) and doses as used in experiments described earlier concerning the induction of endonuclease-sensitive sites (Enninga *et al*, 1986) and cell survival (De Leeuw *et al*, 1994). In these studies, a monochromatic light at 365 nm was used for UVA irradiation (Enninga *et al*, 1986; De Leeuw *et al*, 1994). Therefore, some comparisons are possible. For the induction of endonuclease-sensitive sites in human fibroblasts using UVB and UVA, Enninga *et al* (1986) found a ratio in efficacy (UVB *versus* UVA) of 125. The same high ratio was also found in hamster cells by Kielbassa *et al* (1997). A comparison of the efficacy of MN induction by UVB *versus* UVA in our experiments with fibroblasts, however, revealed a smaller ratio (15 or 25), in FM- or FCM-scored MN, respectively. A similar small yield ratio of 40 per UV dose of UVB and UVA was also found for the induction of dsb by Peak and Peak (1990). One explanation for the smaller ratios of MN induction is that the UVA-induced MN are generated not only from the induced endonuclease-sensitive sites but also from other lesions, e.g., UV-induced dsb (Peak and Peak, 1990). Taken together, these studies highlight the mutagenic relevance of UVA radiation.

A comparison of the MN-inducing effect of UVB, UVA, and γ radiation based on equitoxic doses (D50) is also informative. The D50 for survival of fibroblasts has been reported as 2 Gy of γ rays (Stacey *et al*, 1989), 10 kJ per $m^2$ for UVB (Enninga *et al*, 1986), and 150 kJ per $m^2$ for UVA radiation (Scharffetter *et al*, 1991). The corresponding frequencies for MN are 3%, 1%, and 1%, respectively (**Fig 3**). The 10 kJ per $m^2$ UVB (313 nm) and the 150 kJ per $m^2$ UVA correspond to approximately 1 and 0.2 minimal erythemal dose, respectively, according to the erythemal action spectrum of Parrish *et al* (1982). Such UV doses can be received easily by sun exposure in summer (Wenczl *et al*, 1997), and high doses of UVA can be received from artificial UVA sources such as those employed in tanning salons (Miller *et al*, 1998) or during UVA1 therapy (Stege *et al*, 1996). These doses are highly relevant because it has been shown that 1–2 Gy of γ radiation can cause severe genotoxic damage in cultured human fibroblasts (Stacey *et al*, 1989; Geard and Chen, 1990). Furthermore, it is known that higher cumulative doses can result in skin cancer formation in experimental animals in a relatively short time (Yuspa and Dlugosz, 1991). Several authors have described induction of skin carcinoma several years after X-ray exposure (Traenkle, 1963). The potential mechanism in this case appeared to be the DNA strand breaks that may cause gene rearrangements or karyotypic abnormalities (Yandell *et al*, 1986). It has been reported that a median dose of 12 Gy of X-rays, after a median follow-up period of 42 y of X-ray treated patients, was associated with a 6% incidence of skin cancer (van Vloten *et al*, 1987).

The presented quantitative assessment of the cell-cycle-dependent clastogenic effects of UVB and UVA in skin cells provides us with arguments to suggest that exposure of human skin to UVB as well as to UVA can contribute to loss of heterozygosity as found frequently in several types of human skin cancer cells. Therefore, these lesions are likely to play a role in skin carcinogenesis.

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